

Appl. No. 09/871,961

Art Unit 1631

February 3, 2004

Reply to Non-Responsive Communication of January 22, 2004

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the present application:

Listing of Claims:

1-34. (Canceled)

35. (Previously Presented) A method for diagnosing aspergillosis, comprising steps of:

(a) incubating a body fluid sample from a patient with an ELISA plate having at least one peptide bound thereto;

(b) removing the body fluid sample from the ELISA plate;

(c) incubating the ELISA plate with anti-human IgG/IgE to form peptide-IgG/IgE complexes;

(d) removing IgG/IgE not bound in a complex;

(e) quantitating an amount of peptide-IgG/IgE complexes; and

(f) diagnosing aspergillosis based on the amount of peptide-IgG/IgE complexes,

wherein the at least one peptide is a peptide comprising an amino acid sequence comprising one of SEQ ID NOS: 1-6.

36. (Previously Presented) The method of claim 35, wherein at least one peptide comprises the amino acid sequence of SEQ ID NO: 2.

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37. (Previously Presented) The method of claim 35, wherein the plate is coated with a peptide comprising the amino acid sequence of SEQ ID NO: 2.

38. (Previously Presented) The method of claim 35, wherein the body fluid sample is blood, cerebrospinal fluid, pleural fluid, or saliva.

39. (Previously Presented) The method of claim 35, wherein the body fluid sample is blood.

40. (Previously Presented) The method of claim 35, wherein the IgG/IgE antibody is conjugated to an enzyme and the amount of peptide-IgG/IgE complexes is quantitated by measuring an activity of the enzyme.

41. (Previously Presented) The method of claim 40, wherein the enzyme is peroxidase or alkaline phosphatase, and wherein an enzyme substrate is o-phenylene diamine or nitroblue tetrazolium.

42. (Previously Presented) The method of claim 40, wherein at least one peptide consists of an amino acid sequence of one of SEQ ID NOS 1-6.

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43. (Previously Presented) The method of claim 40, wherein at least one peptide consists of the amino acid sequence of SEQ ID NO. 2.

44. (Withdrawn) A diagnostic kit for aspergillosis, comprising an ELISA plate coated with at least one peptide having an amino acid sequence comprising one of SEQ ID NOS: 1-6.

45. (Withdrawn) The diagnostic kit of claim 44, wherein at least one peptide coating the ELISA plate comprises the amino acid sequence of SEQ ID NO. 2.

46. (Withdrawn) An isolated antibody that specifically binds to a peptide having an amino acid sequence comprising one of SEQ ID NOS: 1-6.

47. (Withdrawn) A method for preventing or treating aspergillosis, comprising:

administering at least one peptide having an amino acid sequence comprising one of SEQ ID NOS: 1-6 to a patient.

48. (Previously Presented) A method for the diagnosis of aspergillosis using at least one peptide containing an amino acid sequence of SEQ ID NOS: 1-6, said method comprising steps of:

- (a) collecting a body fluid sample containing antibodies specific to *Aspergillus fumigatus* (Af-antibodies) from a patient and separating a fluid containing Af-antibodies from any cells present in the fluid;
- (b) incubating the fluid containing Af-antibodies obtained in step a with at least one peptide consisting of an amino acid sequence of SEQ ID NOS: 1-6;
- (c) separating from the resultant incubation mixture residual Af-specific antibodies that do not bind to the at least one peptide in step (b) by centrifugation;
- (d) incubating the residual Af-specific antibodies obtained in step (c) with a mixture of allergens and/or antigens of *Aspergillus fumigatus* coated on a polystyrene ELISA plate to bind antibodies to the ELISA plate;
- (e) washing unbound antibodies from the ELISA plates with an appropriate buffer;
- (f) incubating the washed plates from step e with anti-human IgG/IgE conjugated with an enzyme to obtain immobilized enzyme;
- (g) washing unbound enzyme from the ELISA plates with an appropriate buffer;
- (h) adding a soluble substrate for the enzyme; and

(i) measuring the absorbance values of the wells of ELISA plates in an ELISA reader, wherein the acuteness of aspergillosis is inversely related to the absorbance value.

49. (Previously Presented) The method of claim 48, wherein body fluid may be blood, serum, cerebrospinal fluid, pleural fluids and saliva.

50. (Previously Presented) The method of claim 48, wherein the buffer used is selected from phosphate buffered saline or Tris buffered saline.

51. (Previously Presented) The method of claim 48, wherein the conjugate used is selected from anti-human IgG/IgE peroxidase or anti-human IgG/IgE alkaline phosphatase.

52. (Previously Presented) The method of claim 48, wherein the substrate used is o-phenylene diamine or nitroblue tetrazolium (NBT).

53. (Previously Presented) A method for diagnosing aspergillosis in a patient comprising steps of:

(a) collecting a blood sample comprising *Aspergillus fumigatus* specific antibodies (Af-antibodies) from a patient and separating a serum from the blood;

(b) incubating the patient serum containing Af-specific antibodies with a polystyrene ELISA plate having immobilized thereon at least one peptide consisting of the amino acid sequence of SEQ ID NOS: 1-6 to form an immobilized antibody;

(c) washing the unbound antibodies from the ELISA plate with an appropriate buffer;

(d) incubating the washed plate from step c with anti-human IgG/IgE conjugated with an appropriate enzyme to form an immobilized conjugated enzyme;

(e) washing the unbound conjugated enzyme from the ELISA plate with an appropriate buffer;

(f) adding soluble substrate for the enzyme used in step d; and

(g) measuring the absorbance values of the wells of the ELISA plate, wherein the acuteness of aspergillosis is directly related to the absorbance value.

54. (Previously Presented) The method of claim 53, wherein the body fluid may be blood, serum, cerebrospinal fluid, pleural fluids and saliva.

55. (Previously Presented) The method of claim 53, wherein the buffer used is selected from phosphate buffered saline or Tris buffered saline.

56. (Previously Presented) The method of claim 53, wherein the conjugate used is selected from anti-human IgG/IgE peroxidase or anti-human IgG/IgE alkaline phosphatase.

57. (Previously Presented) The method of claim 53, wherein the substrate used is o-phenylene diamine or nitroblue tetrazolium (NBT).

58. (Withdrawn) A method for diagnosing aspergillosis, comprising:

(a) obtaining mast cells from a patient;

(b) stimulating the mast cells with at least one peptide having an amino acid sequence comprising one of SEQ ID NOS: 1-6;

(c) determining an amount of histamine released by the stimulated mast cells,

(d) diagnosing aspergillosis when the amount of histamine released by the stimulated lymphocytes exceeds a threshold concentration.

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59. (Previously Presented) The method of claim 49, wherein the threshold is 82 nanomolar.

60. (Withdrawn) A method for producing antibodies that specifically bind to *Aspergillus fumigatus* antigens comprising:

(a) administering to a mammal at least one peptide having an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NOS: 1-6;

(b) obtaining serum from the mammal; and

(c) isolating antibodies from the serum that specifically bind to the 18 kD antigen of *Aspergillus fumigatus*.

61. (Withdrawn) A method for proliferating immune cells of a subject comprising culturing cells from blood of the subject in a culture medium comprising at least one peptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6.